

Assessing the impacts of European earthworm invasions in beech-maple
hardwood and aspen-fir boreal forests of the western Great Lakes region

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Assessing the impacts of European earthworm invasions in beech-maple hardwood and aspen-fir boreal forests of the western Great Lakes region

Abstract: In the Great Lakes region, little data exist on the distribution of exotic earthworm species or their relative impacts to soil structure and plant communities in forest types other than those dominated by sugar maple. Our objective was to document the earthworm populations in relation to their proximity to human development (e.g., campgrounds, boat landings, roads) in beech-maple dominated forests in Pictured Rocks National Lakeshore, Michigan, and aspen-fir forests in Voyageurs National Park, Minnesota; and to assess earthworm population relationships with soil characteristics and plant communities. Five earthworm species were commonly found in both forest types in three species assemblages also seen in other forests across the Great Lakes region. Total earthworm biomass supported by beech-maple forests was nearly half that reported in sugar maple dominated forests and the total earthworm biomass supported by aspen-fir forests was half that of the beech-maple forests. Distances to human development were poor predictors of earthworm biomass and species assemblage in general, but were correlated with the presence of *Lumbricus terrestris*. Earthworm richness and biomass were negatively associated with thickness of the O_{litter} and O_e horizons and positively associated with A horizon thickness. Increasing plant richness and changes in plant composition associated with earthworms in the beech-maple forests are likely related to stand conditions that simultaneously affect plant and earthworm populations rather than an earthworm effect. Comparisons to earthworm-free stands were not possible and are needed to further explore any potential causal relationships between earthworm and understory plant populations.

Keywords: boreal forests, cold-temperate hardwood forests, beech, sugar maple, exotic earthworm invasion, aspen-fir, understory plant communities

Introduction

European earthworms (Lumbricidae) are invading previously earthworm-free hardwood forests of the Great Lakes region (Hale et al. 2005^a, Holdsworth et al. 2004, Mortensen and Mortensen 1998, Alban and Berry 1994). In sugar maple (*Acer saccharum*) dominated forests of the Great Lakes region, earthworm invasions have resulted in rapid loss of the forest floor (the accumulated layer of decomposing litter, also referred to as the “duff layer”), development of a thick and dense A horizon, decreased nutrient availability, declines in the diversity and abundance of many native understory plant species and decreased density of tree seedlings (Hale et al. 2005^b, Hale 2004, Bohlen et al. 2004^a, Gundale 2002). Therefore, concerns have been raised about the potential for widespread loss of native forest plant species and the long-term stability of hardwood-forest ecosystems in the Great Lakes region (Frelich et al. 2005).

Earthworm species have diverse feeding preferences and habitat tolerances which can lead to varied impacts when they invade (Hale et al. 2005^b, Bernier 1998, Edwards 1998). In the western Great Lakes region, up to 18 species of European earthworms in four ecological groups have been documented (Reynolds et al. 2002, James 1995, Snider 1991, Bouché 1977). Epigeic species (litter dwelling, e.g., *Dendrobaena octaedra* and *Dendrodrilus rubidus*) are generally small bodied (< 3 cm), pigmented earthworms that live exclusively in the litter layer and feed primarily on the microorganisms found there. Strictly epigeic species, such as *D. octaedra*, are often more tolerant of acidic conditions and are often the only species found in forests dominated by conifer species (McLean and Parkinson 2000, Pearce 1972, Hale personal observations). Epi-endogeic species (generalist surface dwellers) are moderate sized (~3-9 cm), pigmented earthworms that feed on surface litter and burrow in the upper few centimeters of soil but form no persistent burrow system (e.g., *Lumbricus rubellus*). These species are generalist feeders, consuming both organic materials and microorganisms, particularly in the rhizosphere (Hendrix et al. 1999, Bonkowski and Schaefer 1997, Haimi and Boucelham 1991). However, they may be less tolerant of drought conditions when wet refugia are unavailable (Suarez et al. unpublished data, Hale personal observations). Endogeic species (soil dwelling) are generally non-pigmented earthworms that live in the mineral soil horizon (~0-40 cm) forming persistent lateral branching burrow systems (e.g., *Aporrectodea* spp. and *Octolasion* spp.) and range considerably in size (~2–11 cm). Endogeic species feed by ingesting a mixture of mineral soil and soil organic material, including partially decomposed surface litter but do not appear to consume unaltered surface litter (Hale et al. 2005^a, Hendriksen 1990). Anecic species (deep burrowing) are large bodied (>10 cm), generally pigmented earthworms that burrow deeply into the soil horizon, forming persistent unbranching vertical burrows (e.g., *Lumbricus terrestris*) and feed primarily on fresh surface litter. Most earthworms, and anecic species in particular, exhibit strong feeding preferences such that litter with lower C:N ratios are consumed preferentially over litter with higher C:N ratios (e.g. in order of preference basswood > sugar maple > oak > beech > fir > spruce) (Schonholzer et al. 1998, Shipitalo et al. 1988, Satchell and Lowe 1966, Holdsworth et al. unpublished data, Suarez et al. unpublished data). Endogeic and anecic species may be more tolerant of coarse textured and dry soil conditions because they are able to migrate to deeper soil layers and can enter a state of aestivation to avoid desiccation (Edwards and Lofty 1977).

Susceptibility to invasion by earthworms and the magnitude of resulting impacts to native forest communities are expected to be ecosystem specific (Callaham et al. 2005, Vitousek 1990). Forest types vary in the nutritional quality and quantity of litter they produce and the soil types

on which they typically occur (McClaugherty et al. 1985). Therefore, each forest type may be expected to support earthworm populations of different sizes and species composition and the magnitude and nature of the impacts to any given forest type are expected to be related to the traits and size of the earthworm population supported (Hale et al. 2005^b, Bohlen et al. 2004^b, Proulx 2003, Lavelle 1997, Scheu 1987). In the western Great Lakes region, few studies exist on the distribution and abundance of earthworm species or their relative impacts to soil structure and plant communities in forest types other than those dominated by sugar maple. An assessment of the relative threats posed to other important forest types by earthworm invasion is needed so that forest managers in the western Great Lakes region can effectively direct future monitoring, research and management efforts (Hendrix and Bohlen 2002).

Beech-maple (*Fagus grandifolia* and *Acer saccharum*) dominated hardwood forests and boreal mixed hardwood and conifer forests are two widespread forest types in the western Great Lakes region that may be expected to respond differently to invasions by earthworms. In sugar maple dominated forests lacking a beech component, the presence of large amounts of highly digestible and palatable litter on generally well-drained, loamy soils is ideal for the development of large and diverse earthworm populations (Hale et al. 2005^a, Wolters 1999). However, beech-maple dominated forests produce large amounts of less palatable beech litter and can grow on sandy-dry soils near the Great Lakes due to moisture inputs. Less palatable litter and coarse textured soils lead to smaller and less diverse earthworm populations than those seen in sugar maple dominated forests that have no beech component. Boreal forests dominated by aspen (*Populus* spp.), fir (*Abies balsamea*), and spruce (*Picea* spp.) tree species, tend to produce less litter than cold-temperate hardwood forests which is a mixture of palatable and unpalatable litter types. These forests grow in a range of soil conditions but are often present in areas with shallow or rocky soils. Therefore, boreal forests may support even smaller and less diverse earthworm populations, leading to minimal impacts when they invade.

In the western Great Lakes region, a mosaic of earthworm-free and earthworm invaded conditions exists (Holdsworth et al. 2004), where the probability that an area has been invaded is strongly related to the type and intensity of human use. In more rural or remote areas of the region, boat landings, lakeshores, resorts, and roads are often epicenters of earthworm invasion because earthworms are widely used as fishing bait (Holdsworth et al. 2004, Hale personal observations). Therefore, it is possible to use spatial data to identify forest stands with different probabilities of being invaded by earthworms for a comparative study of the impacts of earthworm invasion in important forest types of the region.

We assessed earthworm populations, soil characteristics, and plant communities of beech-maple dominated forests in Pictured Rocks National Lakeshore, Michigan, and of aspen-fir forests in Voyageurs National Park, Minnesota. Our objectives in each forest type were to

- 1) document the distribution, species assemblages, and relative abundance of earthworm populations in relation to their proximity to human development (i.e., campgrounds, boat landings, roads, visitor center) and tree canopy composition;
- 2) document changes in forest floor and upper soil horizons in relation to earthworm populations;

- 3) related diversity, total cover and species composition of understory plant communities to earthworm populations and other environmental factors (i.e. human development, tree canopy composition, soil measures).

Methods

Stand selection and sampling strategy

In both forest types, we selected 20 stands based on GIS analysis of existing data on forest cover, soils, waterways, and landscape features, along with historic maps (Hop et al. 2001) and first person accounts of current and historic human use patterns (Lee Grim, Biologist, Voyageurs National Park; Larry Kallemeyn, Aquatic biologist, U.S.G.S. – Biological Resource Division). Based on previous research (Holdsworth et al. 2004), forest stands within 500 m of probable earthworm introduction points were assigned a high probability of invasion by exotic earthworms, while stands further than 500 m were assigned a low probability of being invaded. We identified 10 aspen-fir stands with low and high probability of being invaded by earthworms in Voyageurs National Park for a total of 20 stands. In the beech-maple forests of Pictured Rocks National Lakeshore, we identified 9 stands with a low probability of being invaded by earthworms and 11 stands with a high probability (Figure 1). In each forest type, stands were selected with similar soils (where possible), disturbance history, age, and canopy composition. Beech-maple stands selected for the study were mature second-growth, unmanaged since the cutover (circa 1900) and had closed, continuous canopies containing a 20-60% beech component. The soils were well-drained ranging from sands to loamy-sands. Aspen-fir stands selected for the study were mature, had not been harvested for ≥ 30 years, had continuous to discontinuous canopies ranging from 60-100% total cover and $\geq 50\%$ dominance by aspen. The soils generally ranged from loam to a silty clay loam, but shallow bedrock, gravel, and boulders were common throughout. Within each stand, three 10 x 10 m vegetation plots were established, and are described in greater detail below. Surveys of forest plant communities, soils, and earthworm communities were conducted June 16-29, 2004 in Pictured Rocks and July 8-26, 2004 in Voyageurs.

Earthworm populations

Earthworm populations were surveyed in three 0.12 m² (35 cm x 35 cm) subplots established randomly within each 10 x 10 m vegetation plot using liquid extraction and midden counts (Hale et al. 2005^a, Lawrence and Bowers 2002). Because *L. terrestris* middens (piles of cast material surrounding the burrow entrance) are visually distinctive and each burrow is generally occupied by one individual, midden counts reliably estimate the only anecic species in this region (Edwards 1998). Following the midden count, each subplot was sampled using a liquid extraction solution of 40 g ground yellow mustard to 4 l of water. The liquid mustard extraction method has been shown to be as or more reliable than the hand sampling method for determining relative abundance for epigeic, endogeic and anecic species of earthworms (Hale et al. 2005^a, Zaborski 2003). All earthworms collected were killed in 70% isopropyl alcohol, preserved in 10% formalin, and identified using Schwert (1990) and Reynolds (1977). Ash-free dry biomass (g) was estimated from the length (mm) of each preserved specimens using allometric equations (Hale et al. 2004). Biomass of earthworms detected in the midden counts but not collected, was estimated as the average biomass of all *L. terrestris* specimens collected in

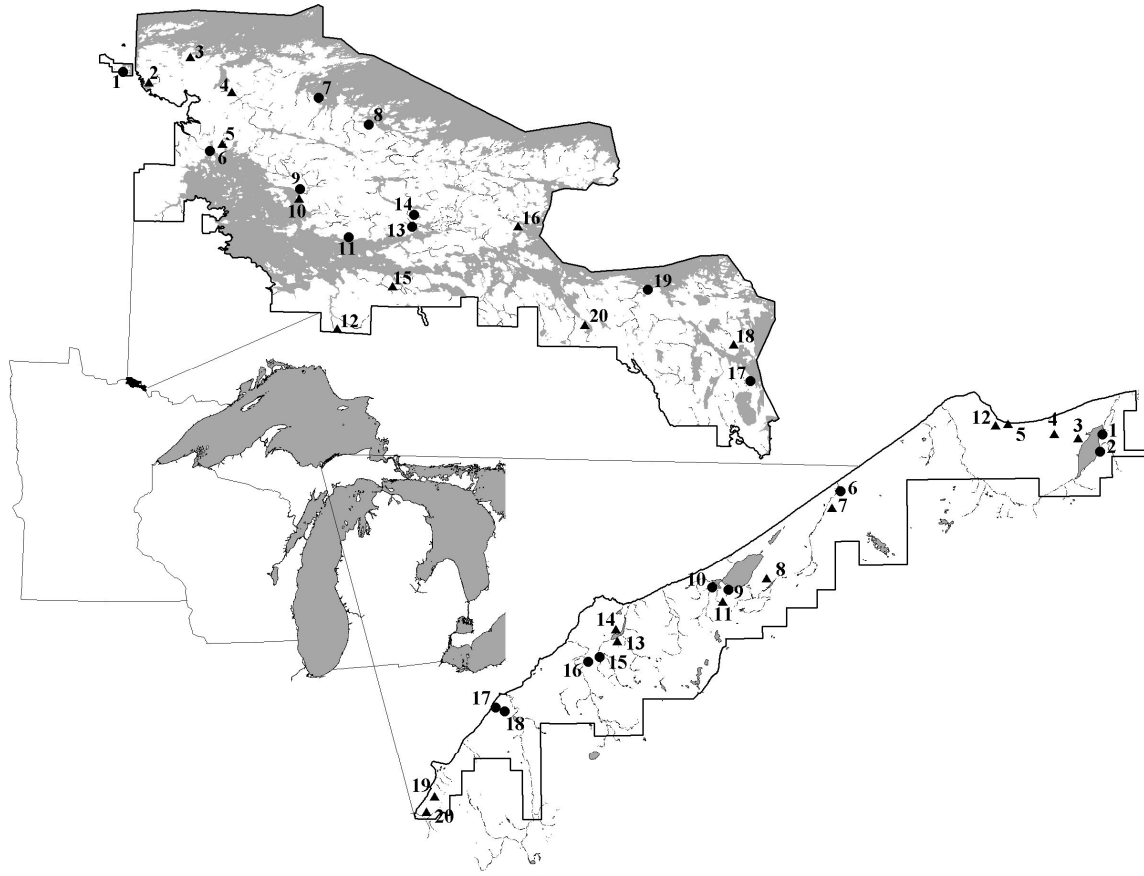


Figure 1. Aspen-fir forest stands in Voyageurs National Park (top), Minnesota and beech-maple forest stands in Pictured Rocks National Lakeshore (bottom), Michigan. Symbols represent the predicted probability of earthworm invasion for each stand, where circles indicate high probability and triangles indicate low probability.

that forest type. For each stand, mean total earthworm biomass (AFDg/m^2), species specific biomass, and earthworm species richness were each calculated as the grand mean of the three plot means.

In sugar maple dominated forests, 6 common earthworm species assemblages have been identified that reflect the life history traits of the different earthworm species and the probability that any given species has access to a stand (Hale et al. 2005^a, Holdsworth et al. 2004). For example, the strictly epigeic *D. octaedra* is often found alone because it can live in an unaltered, thick forest floor environment and spreads rapidly due to its parthenogenic nature (Dymond et al. 1997, Tiunov et al. unpublished). However, endogeic species, such as *Aporrectodea* spp., appear unable to utilize unaltered forest floor material and have not been found in the absence of one or more epigeic or epi-endogeic species (i.e. *D. octaedra*) (Hale et al. 2005^a). Further, the effects of different earthworm assemblages can be independent of total biomass and diversity (Hale et al. 2005^b). Therefore, stands were assigned to one of the 6 assemblages based on the presence/absence of different earthworm species. Species assemblage 1 contains strictly epigeic species only (i.e. *D. octaedra*, *D. rubidus*). Species assemblage 2 contains epigeic and epi-

endogeic species (e.g. *L. rubellus* (adults), *Lumbricus* (juveniles), and *D. octaedra*). Species assemblage 3 contains epigeic and endogeic species (e.g. *Aporrectodea* spp., *O. tyrtaeum*, *D. octaedra*). Species assemblage 4 contains epigeic, epi-endogeic and endogeic species (e.g. *Aporrectodea* spp., *Dendrobaena octaedra* and *L. rubellus*). Species assemblage 5 contains epigeic, endogeic and anecic species (e.g. *Aporrectodea* spp., *Dendrobaena octaedra*, *L. terrestris*). Species assemblage 6 contains species from all ecological groups epigeic, epi-endogeic, endogeic and anecic species (e.g. *Aporrectodea* spp., *Dendrobaena octaedra*, *L. rubellus*, *L. terrestris*).

Distance to human development

We used GIS to calculate the distance (m) of each plot to adjacent cover types, lakes and water ways, roads, trails, and campsites. For each stand, mean distances to human development ($n = 3$, mean of plot distances) were calculated to the next vegetation type, to the nearest trail, road, campsite, lake, stream, and when possible to the 2nd and 3rd nearest lake and stream.

Tree canopy composition

In each stand, three 10 x 10 m plots were randomly located ≥ 50 m from each other, the forest type edge, a lakeshore, and inclusions (i.e., wetlands). GPS coordinates were collected at the center of each 10 x 10 m plot. Overstory tree species (≥ 10.0 cm dbh) and dbh were recorded using a 10 ft²/acre (2.3 m²/ ha) wedge prism at the center of each 10 x 10 m plot. Total canopy cover was visually estimated at the plot center. Mean total basal area (m²), species specific basal area, tree density, species richness, and Shannon diversity were calculated for each stand ($n = 3$).

Forest floor and upper soil horizons

The presence and thickness of the forest floor (O_{litter} , O_e and O_a horizons) and upper soil horizons (A and/or E horizons) were measured by extracting nine soil cores, 6 cm diameter and 15 cm deep, randomly located within each 10 x 10 m plot. For each horizon present in each core the color (chroma, value, and hue), texture class (1 = sand to 8 = silty-clay) and percent gravel class (1 = 0-15%, 2 = 15-35%, 3 = 35-65%) were recorded (GretagMacbeth 2000, Buol et al. 1989). Mean horizon thickness (cm) and color, texture and percent gravel classes for each stand ($n = 3$) was calculated as the grand mean of the three plot means ($n = 9$) in each stand.

The forest understory plant community

In each 10 x 10 m plot, the understory vascular plant community was surveyed in four structural-lifeform layers (Almendinger 1991) including 1) herbaceous plants; 2) tree seedlings and shrubs ≤ 0.5 m tall; 3) small saplings and shrubs > 0.5 m tall and ≤ 2.0 cm diameter at breast height (dbh); and 4) large saplings and shrubs > 2.0 cm and < 10.0 cm dbh. In each structural-lifeform layer, we identified all plant species (Gleason and Cronquist 1991), estimated percent cover of all plant species, and counted stems of tree and shrub species (Almendinger 1991). Plant species richness, Shannon diversity and total percent cover were calculated for each layer and for the understory plant community as a whole using pseudo-species (e.g. *Acer rubrum* seedling, *A. rubrum* small saplings and *A. rubrum* large saplings) for the same plant species present in multiple layers. Stand means of percent cover, stem counts, richness, and Shannon diversity were calculated as the grand mean ($n = 3$) of the three plot means ($n = 9$) for each layer and the understory plant community as a whole.

Analytical methods

For each forest type, independent analyses were conducted. For purposes of statistical analysis, earthworm biomass data were log transformed. A log-transformation technique described in McCune and Grace (2002) was used. The transformed value (b_{ij}) equals:

$$b_{ij} = \log_{10}(x_{ij} + d) - c$$

Where c = integer of ($\log_{10}(\min(x))$), $\min(x)$ = lowest non-zero x and d = inverse $\log_{10}(c)$.

This transformation technique was appropriate for the earthworm data set because it preserves both zero values and differences in magnitude that exist in the data set. Natural log transformations of distance measures to human development and O horizon and A horizon thickness measures were used as needed to normalize distributions for analysis. No transformations were required for plant species richness or Shannon diversity measures. To normalize distributions, all plant percent cover measures were converted to ordinal cover classes (i.e. 1 = 0 - <1%, 2 = 1 - <5%, 3 = 5 - <25%, 4 = 25 - <50%, 5 = 50 - <75%, 6 = 75 - 100%).

To address our first objective, relating earthworm distribution, species assemblages and abundance to human development and overstory composition, we used a combination of paired t -tests, ANOVA, linear regression analyses and multivariate ordination analyses (McCune and Grace 2002, SAS 2001, McCune and Mefford 1999, Montgomery and Peck 1992). The number and biomass of different earthworm species and assemblages, and the total biomass of a given assemblage were compared between forest types using paired t -tests and ANOVA. One-way ANOVA was used to compare earthworm biomass and richness among the different earthworm species assemblages within and between the two forest types. Within each forest type, paired t -tests were used to test for differences in mean earthworm biomass and richness between stands assigned to high and low probability of being invaded and differences in species assemblages were described. To test the relationships of earthworm biomass, richness and species assemblages to the distances to human development (i.e. nearest vegetation type, trail, road, campsite, lakes, streams) and tree canopy characteristic (i.e. total basal area, species specific basal area, tree density, species richness and Shannon diversity) a combination of multiple and simple linear regression analysis was used.

Tree canopy composition and earthworm community composition among stands were assessed using non-metric multidimensional scaling (NMS) ordination with Sorenson's distances in PC-ORD (McCune and Grace 2002, McCune and Mefford 1999). Preliminary NMS analysis with 50 runs of real data and 100 runs with randomized data for a Monte Carlo test of significance were conducted. The optimal number of ordination dimensions was determined based on the lowest stress achievable with the fewest number of dimensions. A series of secondary NMS analyses were conducted with the designated number of ordination dimensions, 50 runs of real data, and 100 runs with randomized data. If consistent, non-random results after multiple runs from a random starting configuration were found, the secondary analysis with the lowest stress and final instability was used as the starting configuration for the final NMS analysis reported.

Additionally, the multi-response permutation procedure (MRPP), which tests the hypothesis of no difference between two or more groups, was used (with Sorenson's distances)

to compare overstory composition among stands with different earthworm assemblages (PC-ORD, McCune and Mefford 1999). The MRPP is a non-parametric procedure that compares the observed weighted mean within-group distance (δ) to the expected distribution of δ (McCune and Grace 2002, Mielke and Berry 2001). A p -value is generated ($\alpha = 0.05$), indicating the probability that the observed difference is due to chance, and an agreement statistic (A) that indicates the degree to which groups differ from that expected by chance. The highest possible value for $A = 1$ when all species are identical within groups and $A = 0$ when species differences equal those predicted by chance.

Our second objective was to relate changes in the forest floor and upper soil horizons to earthworm populations. Analysis of these relationships included simple linear regression to test the relationships of earthworm biomass and richness to the thickness of the forest floor (i.e. O_{litter} , O_e , O_a and total O horizons) and upper soil horizons (i.e. A and E) (Montgomery and Peck 1992, SAS 2001). Because earthworm assemblages and species richness were completely confounded with increasing earthworm biomass, no explicit analysis of relationships to earthworm assemblages or earthworm richness was completed.

To address our final objective, comparing diversity, cover, and species composition of understory plant communities in relation to earthworm populations and other environmental factors (i.e. human development, tree canopy composition, soil measures), we used a combination of linear regression and multivariate ordination analyses (McCune and Grace 2002, SAS 2001, McCune and Mefford 1999, Montgomery and Peck 1992). In each structural layer and for the understory community as a whole, simple linear regression was used to test the relationships of earthworm biomass to plant species richness, Shannon diversity, and total cover (Montgomery and Peck 1992, SAS 2001).

To assess potential relationships among earthworm populations and other environmental factors (i.e. human development, tree canopy composition, soil measures) and understory plant community composition ordination analysis was used. Plant communities in each structural life-form layer (i.e. large saplings and shrubs, small saplings and shrubs, seedling, and herbaceous) and the understory community as a whole were ordinated using non-metric multidimensional scaling (NMS) ordination with Sorenson's distances in PC-ORD (McCune and Grace 2002, McCune and Mefford 1999) as described for objective 1 above. Ordinations were conducted with the rarest plant species ($n \leq 3$) deleted. Environmental variable joint-plot overlays of the understory plant community ordinations were used to assess the relationships of earthworm biomass and species assemblage, distances to human development, canopy tree species basal area, and soil horizon thickness and texture measures to the plant communities.

Results

Earthworm species, biomass and species assemblages in beech-maple and aspen-fir forests

Five species of earthworms and juveniles of the genus *Lumbricus* were found in multiple stands of both forest types (Table 1). It is impossible to separate juvenile *Lumbricus rubellus* and

Table 1. Earthworm taxonomic groups present in each forest type including the number of stands each species was detected (total of 20 stands in each forest type) and the mean (S.E.) number and biomass (ash-free dry grams / m²) of each species when present in a stand.

Earthworm taxonomic group	<u>beech-maple forests</u>			<u>aspen-fir forests</u>		
	stands present	mean N	mean biomass	stands present	mean N	mean biomass
<i>Aporrectodea</i> spp. (includes <i>A. caliginosa</i> , <i>A. tuberculata</i> and <i>A. juveniles</i>)	11	3.7 ^a (0.8)	1.9 ^a (0.5)	7	0.8 ^b (0.2)	0.4 ^b (0.1)
<i>Dendrobaena octaedra</i>	18	3.9 (1.1)	0.2 (0.05)	19	2.8 (0.5)	0.1 (0.02)
<i>Dendrodrilus rubidus</i>	0	0	0	1	1	0.1
<i>Eiseniella tetraedra</i>	0	0	0	1	1	0.1
<i>Lumbricus rubellus</i> (adults)	7	0.3 ^a (0.05)	0.1 (0.03)	6	0.7 ^b (0.2)	0.5 (0.2)
<i>Lumbricus terrestris</i> (adults)	6	0.7 (0.2)	2.6 ^a (0.7)	2	0.3 (0.1)	0.8 ^b (0.2)
<i>Lumbricus</i> juvenile *	11	3.1 (0.8)	1.3 (0.4)	8	1.4 (0.5)	0.7 (0.3)
<i>Octolasion tyrtaeum</i>	1	3	0.6	0	0	0

* when it was impossible to identify juveniles of a given genus to species, they are listed separately

^{a,b} different letters indicate significantly different ($p \leq 0.10$) number or biomass of the species between forest types.

L. terrestris. Therefore, *Lumbricus* juveniles must be treated as a separate taxonomic group (Table 1). Earthworm species in the genus *Aporrectodea* (adults and juveniles) were combined into a single taxonomic group because of difficulty in distinguishing species and shared life history traits (Table 1). In addition, *Dendrodrilus rubidus* and *Eiseniella tetraedra* were found as single individuals in two different aspen-fir stands and three small individuals of *Octolasion tyrtaeum* were found in a beech-maple stand (Table 1). Due to the rarity of *E. tetraedra* (four other state records, Reynolds et al. 2002) it was excluded from the species assemblage analysis. *D. rubidus* was combined with *D. octaedra* because they have very similar life history traits. Similarly, the endogeic species *O. tyrtaeum* was combined with the *Aporrectodea* spp. taxonomic group.

In the beech-maple forest type, the mean numbers and biomass of *Aporrectodea* spp. and the mean number of *L. terrestris* (adults) were more than 3 times higher than in the aspen-fir forest type. There were more than twice the mean number of *L. rubellus* (adults) in the aspen-fir forest type than in the beech-maple, but the biomass was the same in both (Table 1).

Three earthworm assemblages (1, 4 and 6 as described in methods) were present in both forest types (Table 2). One assemblage contained only the small-bodied, strictly litter dwelling

(epigeic) species *D. octaedra*. A second assemblage contained three species including litter dwelling *D. octaedra*, soil dwelling (endogeic) *Aporrectodea* spp. and the epi-endogeic species *L. rubellus* and *L. juveniles*. The third assemblage contained *D. octaedra*, *Aporrectodea* spp., *L. rubellus* and also contained the large-bodied, deep burrowing (anecic) species *L. terrestris*. The particular assemblages found were confounded with increasing earthworm diversity and total biomass (Table 2). Total earthworm biomass for each assemblage was similar between forest types (Table 2).

Table 2. Earthworm species assemblages in each forest type including the number of stands with each assemblage and the mean (S.E.) of total earthworm biomass (AFDg/m²) for each assemblage.

Earthworm assemblages	<u>beech-maple forests</u>		<u>aspen-fir forests</u>	
	N stands	mean biomass [*]	N stands	mean biomass [*]
earthworm-free	2	0.0 ^a	1	0.0 ^a
<i>Dendrobaena octaedra</i>	7	0.13 ^b (0.05)	9	0.15 ^b (0.03)
<i>D. octaedra</i> , <i>Aporrectodea</i> spp., <i>L. rubellus</i> (adults) and <i>L. juvenile</i>	5	1.36 ^c (0.50)	8	0.86 ^c (0.17)
<i>D. octaedra</i> , <i>Aporrectodea</i> spp., <i>L. rubellus</i> (adults), <i>L. juvenile</i> and <i>L. terrestris</i> (adults)	6	7.99 ^d (1.60)	2	3.53 ^d (1.66)

^{*} total earthworm biomass of the same assemblages was not significantly different between forest types.

^{a,b,c,d} different letters indicate significantly different ($p \leq 0.003$) total earthworm biomass among earthworm assemblages within and between forest types.

Earthworm populations in relation to human development and canopy composition

In both forest types, earthworms were located in all stands assigned a high probability of being invaded by earthworms. However, of the stands assigned low probability of being invaded (i.e. high probability of being earthworm-free) only one aspen-fir stand and two beech-maple stands were earthworm-free (Figure 2). Because of the low number of earthworm-free stands, comparisons between invaded and uninvaded stands were not possible. However, in the aspen-fir forest type, stands assigned a high probability of being invaded had higher mean total earthworm biomass ($n = 10$, mean = 1.1 AFDg/m², S.D. = 1.5) than those assigned a low probability of being invaded ($n = 10$, mean = 0.4 AFDg/m², S.D. = 0.5; $p = 0.08$, $\alpha = 0.10$). In beech-maple forests, due to high variability in earthworm biomass across all stands, mean total earthworm biomass was not significantly different in stands assigned high ($n = 9$, mean = 4.6 AFDg/m², S.D. = 5.1) versus low ($n = 11$, mean = 1.3 AFDg/m², S.D. = 2.4) probability of being invaded. In both forest types, one species of earthworm, *L. terrestris* (nightcrawler), was present almost exclusively in stands assigned a high probability of being invaded. In beech-maple forests, 5 of the 9 high probability stands contained *L. terrestris* compared while only one of the 11 low

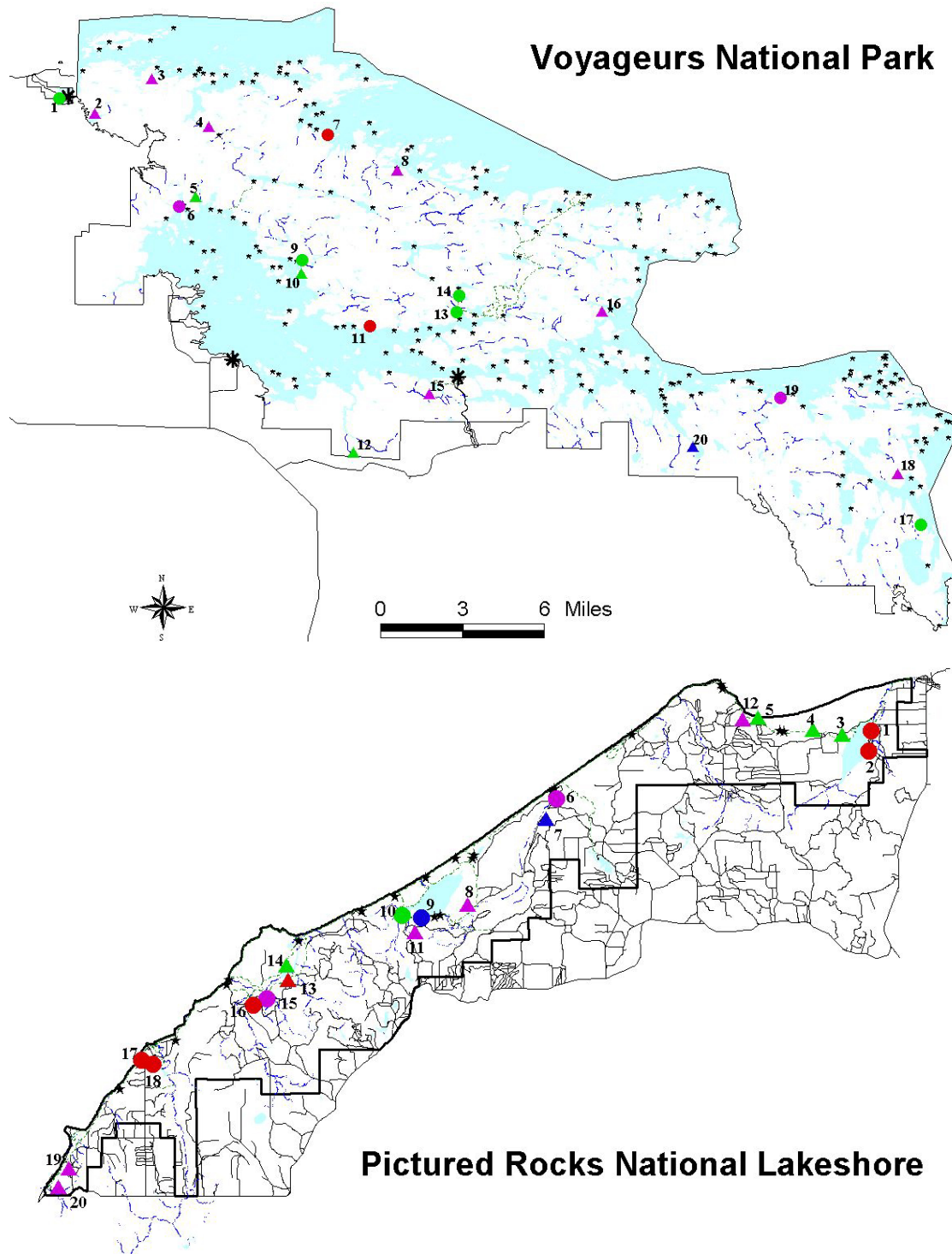


Figure 2. Aspen-fir forest stands in Voyageurs National Park, Minnesota and beech-maple forest stands in Pictured Rocks National Lakeshore, Michigan in relation to earthworm species assemblages and human development. Symbol shape represents the predicted probability of earthworm invasion for each stand, where circles indicate high probability and triangles indicate low probability. Symbol color indicates the earthworm species assemblage where blue = earthworm-free, pink = *Dendrobaena octaedra* only, green = *D. octaedra*, *Aporrectodea* species and/or *Lumbricus rubellus*, *Lumbricus* juveniles, and red = *L. terrestris* and a mixture of all other species. Human developments indicated include visitor centers (black asterisk), campgrounds (black star), trails (green lines), roads (thin black line), streams (blue lines) and lakes (light blue).

probability stands had *L. terrestris*. In aspen-fir forests, only high probability stands contained *L. terrestris*.

In simple and multiple linear regression analysis, the distance to human development (i.e., nearest vegetation type, trail, road, campsite, lakes, streams) were poor predictors of total earthworm biomass, species richness, and species assemblage. Similarly, tree canopy characteristics (i.e. total basal area, species specific basal area, tree density, species richness, and Shannon diversity) had no relationship with total earthworm biomass, species richness or assemblages.

Overstory composition was similar among all stands in each forest type. Ordination analysis (NMS) of canopy composition and earthworm community composition yielded no significant patterns among stands. In MRPP analysis, differences in overstory composition among stands with different earthworm assemblages were small ($A \leq 0.05$; $p \geq 0.10$).

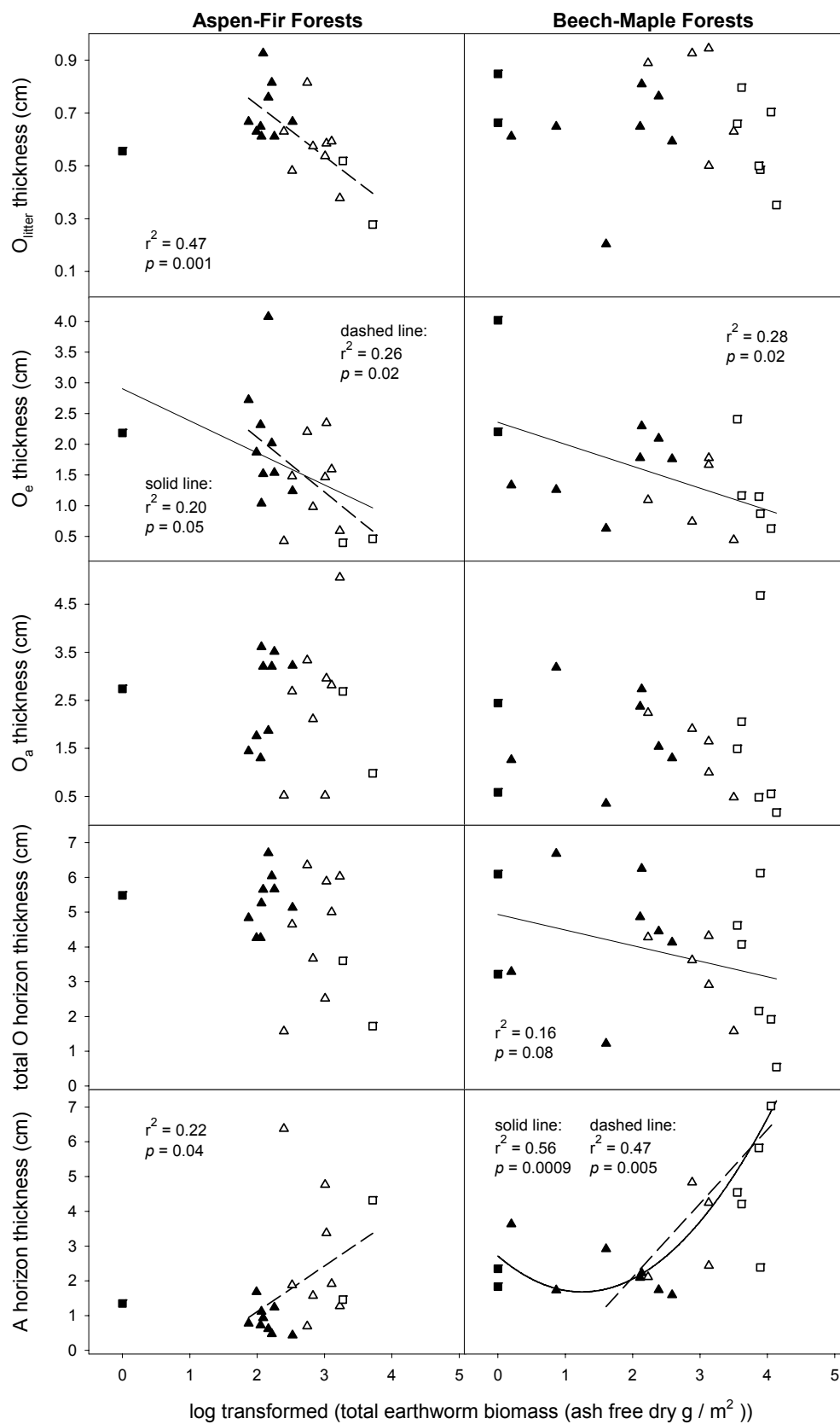
Forest floor and upper soil horizons in relation to earthworm populations

In the aspen-fir forest type, O_{litter} and O_e thickness decreased with increasing total earthworm biomass (Figure 3). However, O_a and total O horizon thickness had no relationship with total earthworm biomass. In the beech-maple forest type, the O_e and total O horizon thickness decreased with increasing total earthworm biomass (Figure 3). In both forest types, A horizon thickness increased with total earthworm biomass in a non-linear fashion (Figure 3). At low earthworm biomass (< 0.10 AFDg/m²) A horizon thickness did not change. However, above total earthworm biomass of approximately 0.10 AFD grams/m² (~ 2.0 log transformed values) A horizon thickness increased linearly with earthworm biomass (Figure 3).

Plant species richness, diversity, and cover in relation to earthworm populations

In the aspen-fir forest type, combined herbaceous plant and tree seedling richness and herbaceous plant total percent cover increased weakly with total earthworm biomass and earthworm diversity (Figure 4) while Shannon diversity had no relationship with earthworm biomass. In the beech-maple forest type, herbaceous plant richness and Shannon diversity increased with total earthworm biomass (Figure 4), but total percent cover had no relationship with earthworm biomass. For both forest types, total earthworm biomass was unrelated to plant species richness, Shannon diversity, and total percent cover for the seedling, small sapling and shrub, and large sapling and shrub structural life-form layers and the understory community as a whole.

Figure 3 (page 13). Scatter plots of O and A horizon thickness and log transformed total earthworm biomass. Solid lines indicate significant fitted relationships including all stands in each forest type ($n = 20$). For the aspen-fir forests, dashed lines indicate significant linear relationships with the earthworm-free outlier stand excluded. For the beech-maple forests, the dashed line indicates a significant linear relationship with the five data points below total earthworm biomass of 2 excluded. Symbols indicate the earthworm species assemblage where solid squares = earthworm-free, solid triangles = *Dendrobaena octaedra* only, empty triangles = *D. octaedra*, *Aporrectodea* species and/or *Lumbricus rubellus*, *Lumbricus* juveniles, and empty squares = *L. terrestris* and a mixture of all other species.



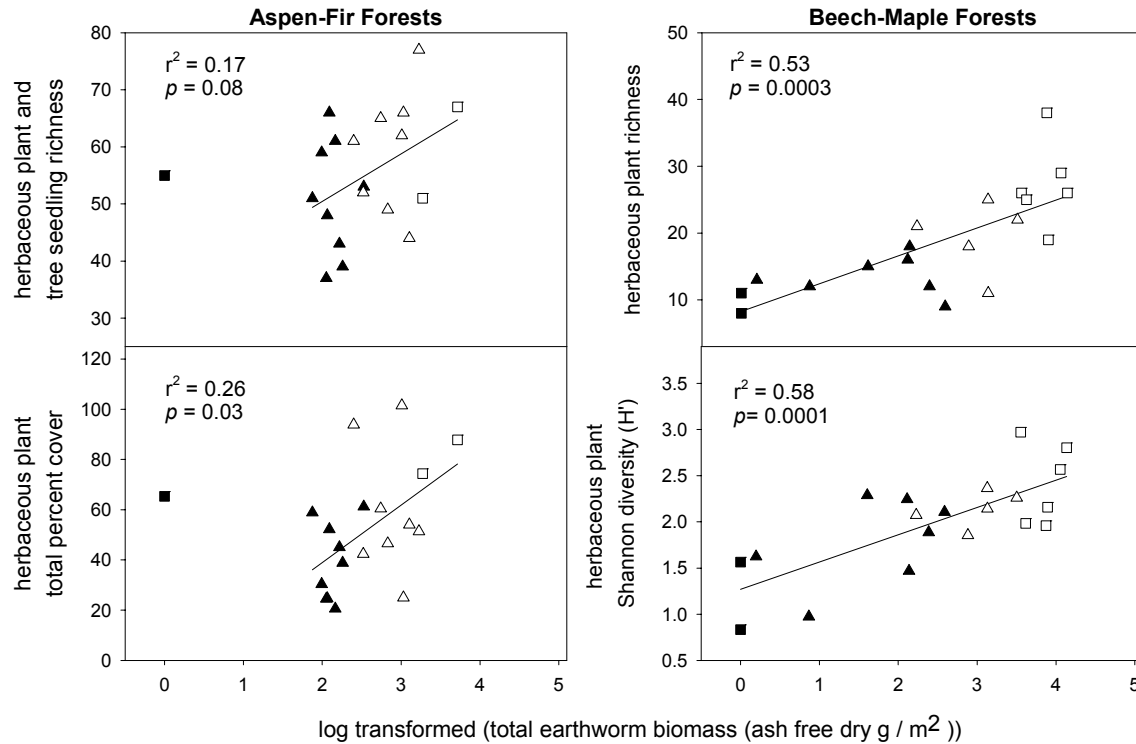


Figure 4. Scatter plots of herbaceous plant richness, Shannon diversity and total percent cover and log transformed total earthworm biomass. Solid lines indicate significant fitted relationships including all stands in the beech-maple forest type ($n = 20$) and the earthworm-free stand is excluded in the aspen-fir forest type ($n = 19$). Symbols indicate the earthworm species assemblage where solid squares = earthworm-free, solid triangles = *Dendrobaena octaedra* only, empty triangles = *D. octaedra*, *Aporrectodea* species and/or *Lumbricus rubellus*, *Lumbricus* juveniles, and empty squares = *L. terrestris* and a mixture of all other species.

Understory composition in relation to earthworm populations and other environmental factors

NMS ordination of the understory plant community as a whole (herbs, seedlings, small saplings and shrubs, large saplings and shrubs combined) in both forest types, resulted in an optimal three-dimensional solution (final stress ≤ 11.821 , final instability = 0.00003) with cumulative proportion of variance represented by the three-dimensional ordination of $r^2 = 0.883$ and 0.849 for beech-maple and aspen-fir, respectively. Monte Carlo tests resulted in final stress values in the real data (means for axis 1=46.2, axis 2=19.4, axis 3=12.1; axis 1=47.9, axis 2=17.4, axis 3=10.8 for aspen-fir and beech-maple, respectively) well below the range of those of the randomized data set (means for axis 1=50.3, axis 2=27.3, axis 3=18.2; axis 1=50.5, axis 2=27.7, axis 3=18.5 for aspen-fir and beech-maple, respectively; $p \leq 0.009$) indicating that the ordination is significantly different than that expected by chance.

In the beech-maple forest type, ordination Axis 1 represented nearly half of the observed variability in the understory plant community ($r^2 = 0.481$) and was associated with a gradient of increasing herbaceous plant richness, Shannon diversity and total percent cover (Figures 5 and 6). Axis 2 ($r^2 = 0.261$) represented a gradient from high large sapling cover and low herbaceous plant cover to low large sapling cover and high herbaceous plant cover. Axis 3 ($r^2 = 0.142$) represented a gradient from low to high small sapling cover. The combination of axes 1 and 2

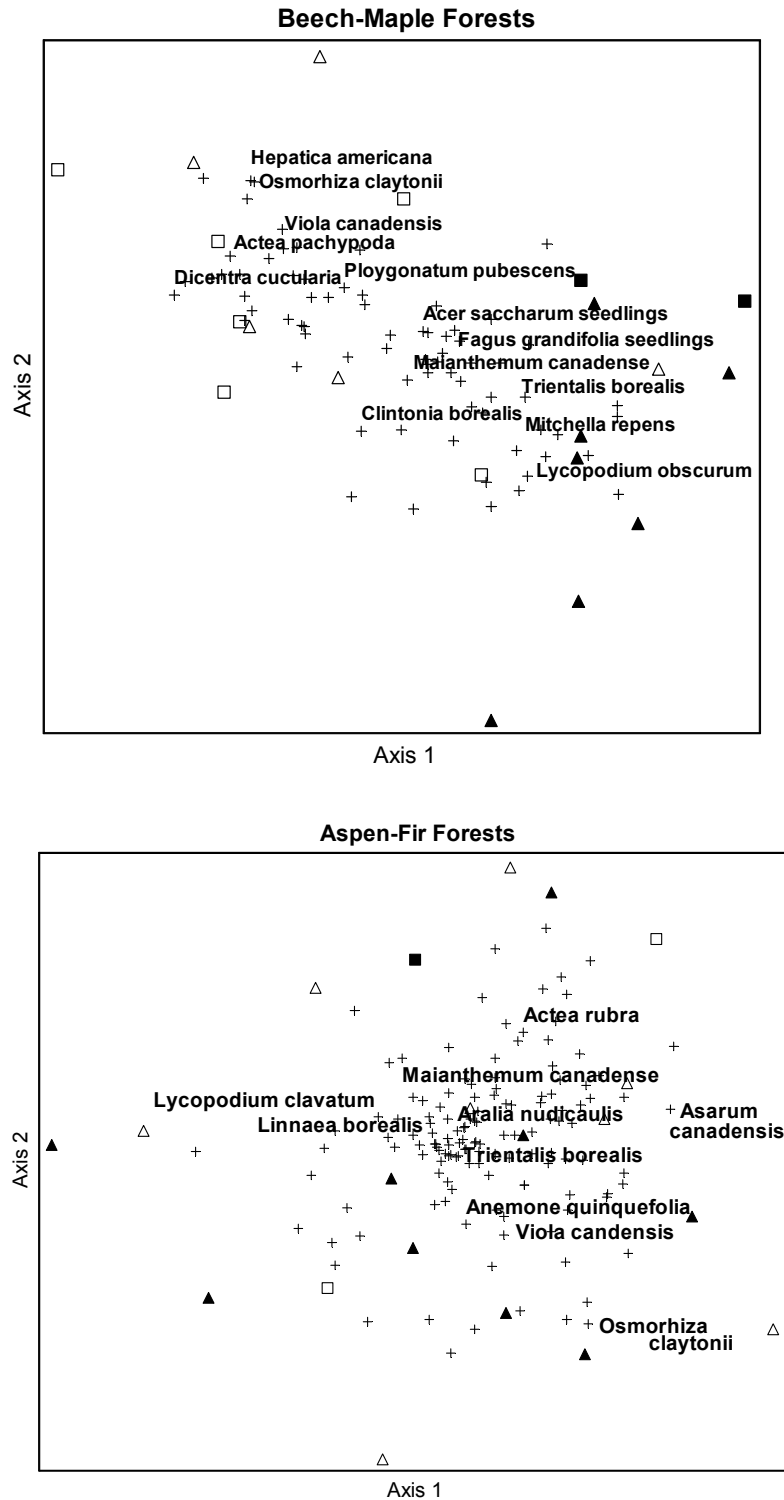


Figure 5. Ordination of understory plant communities in each forest type. Axis 1 and 2 cumulative $r^2 = 0.742$ and 0.659 in beech-maple and aspen-fir forest stands, respectively. Selected plant species names listed adjacent to the plant species symbol (+). Symbols indicate the earthworm species assemblage of each stand where solid squares = earthworm-free, solid triangles = *D. octaedra* only, empty triangles = *D. octaedra* with *Aporrectodea* species and/or *L. rubellus*, and empty squares = *L. terrestris* with one or more of the other species.

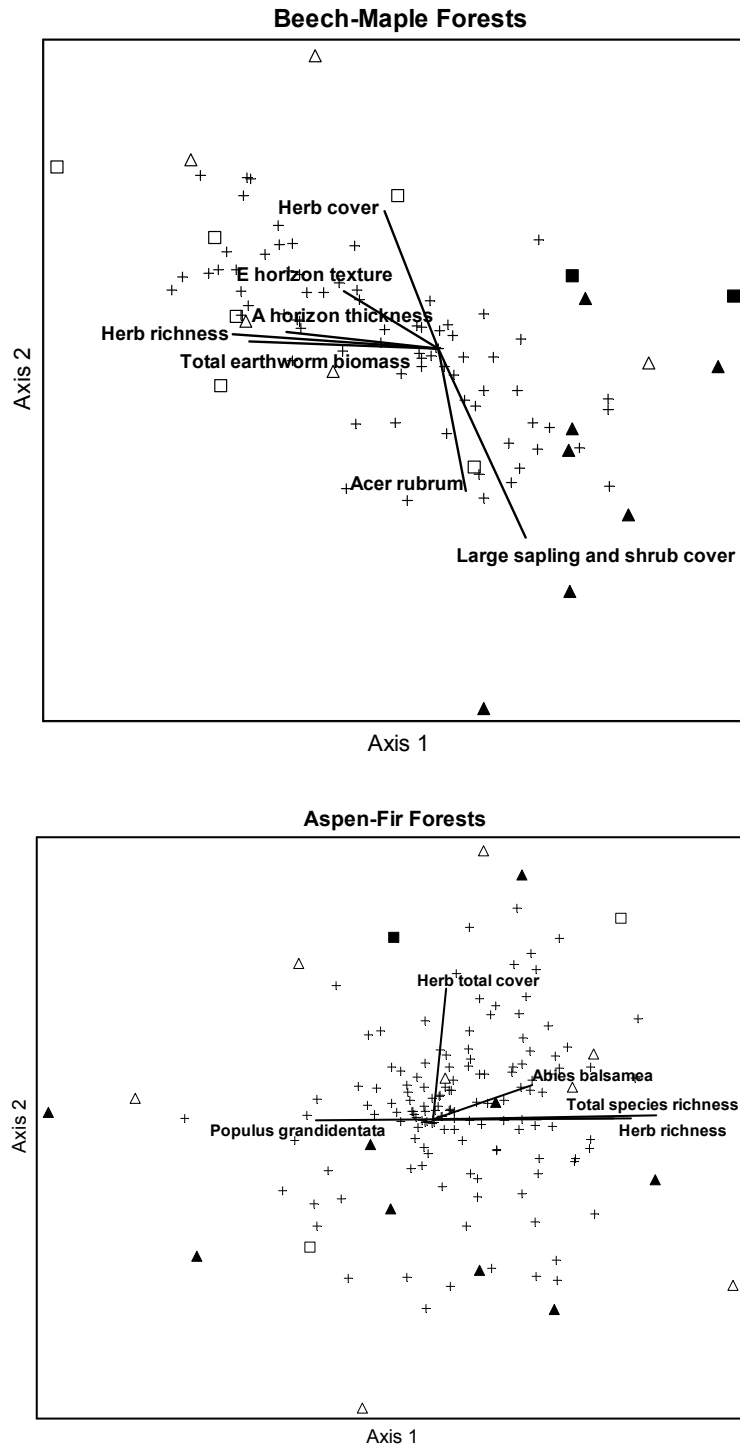


Figure 6. Ordination of understory plant communities in each forest type with joint plot overlay of vectors of selected environmental variables ($r^2 \geq 0.300$). Symbols indicate the earthworm species assemblage of each stand where solid squares = earthworm-free, solid triangles = *D. octaedra* only, empty triangles = *D. octaedra* with *Aporrectodea* species and/or *L. rubellus*, and empty squares = *L. terrestris* with one or more of the other species.

(cumulative $r^2 = 0.742$), represents a gradient in the understory plant community from one of high seedling and sapling diversity and cover with low herbaceous plant diversity, typified by species such as *Lycopodium obscurum*, *Clintonia borealis*, *Mitchella repens* and *Trientalis borealis* (Figure 5, Appendix 1); to an understory plant community of low sapling diversity and cover with high herbaceous plant richness and cover, typified by species such as *Osmorhiza claytonii*, *Actaea pachypoda*, *Hepatica americana*, and *Viola canadensis* (Figure 5, Appendix 1). In joint-plot overlays of environmental variables (earthworm biomass, structural life-form plant species richness, Shannon diversity and total percent cover, O and A horizon thickness, E horizon soil texture, stand slope and aspect, distances to human development, and tree species basal area), ordination Axis 1 was associated with increasing total earthworm biomass and richness ($r^2 = 0.558$ and 0.567 , respectively), loamy soil texture in the E horizon ($r^2 = 0.279$), O_e horizon thickness ($r^2 = 0.376$, no shown) and A horizon thickness ($r^2 = 0.45$) (Figure 6, Appendix 2). Along Axis 2, herbaceous plant cover ($r^2 = 0.406$) was inversely associated with increasing large sapling and shrub cover ($r^2 = 0.558$) and *Acer rubrum* basal area ($r^2 = 0.289$) (Figure 6, Appendix 2). Axis 3 was associated with increasing distances to lakes and streams ($r^2 \geq 0.248$). Ordination axes had no strong correlations with Shannon plant diversity, stand slope and aspect, distances to vegetation types, roads, trails, campsites, or any other canopy tree species (Figure 6, Appendix 4).

In the aspen-fir forest type, ordination Axis 1 represented over a third of the observed variability in the understory plant community ($r^2 = 0.380$) and was associated with a gradient of increasing herbaceous plant and tree seedling richness (Figures 5 and 6, Appendix 3). Axis 2 ($r^2 = 0.279$) represented a gradient of increasing herbaceous plant cover. Axis 3 ($r^2 = 0.189$) exhibits no evident pattern. In joint-plot overlays of environmental variables, axis 1 was related to total plant species richness ($r^2 = 0.652$), herbaceous plant richness ($r^2 = 0.529$) and *Abies balsamea* basal area ($r^2 = 0.289$). Ordination axes had no strong correlations with earthworm biomass or richness, Shannon plant diversity, O and A horizon thickness, E horizon soil texture, stand slope and aspect, distances to human development, or any other canopy tree species (Figure 6, Appendix 4).

Discussion

Predicting earthworm invasions across the landscape

While proximity to human development was a poor predictor of whether or not any earthworms were present, it was correlated with the presence of *L. terrestris* and the most diverse earthworm assemblages (Figure 2). Exotic earthworms were more widely distributed across both forest types than had been predicted by their proximity to human development during stand selection. While our predictions of high vs. low probability of being earthworm invaded were less than successful and we located only 1 or 2 earthworm-free stands, we did manage to document a range of earthworm biomass and assemblages. Further, the high probability stands also were more likely to contain the largest and most diverse earthworm assemblages identified by the presence of the common nightcrawler, *L. terrestris*, (Figure 2, Table 2).

Our ability to predict earthworm invasions across the landscape may be improved by the development of metrics that include not only the presence of human developments but also the intensity and duration of use, and important environmental characters (e.g. forest type, soil

texture, soil moisture). While intensity of use is often associated with the human developments we examined (i.e., roads, lakeshores, etc.), in Pictured Rocks (beech-maple forests) roads are common throughout the park but the level of use ranges widely and another measure of intensity of use may be needed to more clearly delineate areas with high versus low probability of earthworm invasion. In Voyageurs National Park (aspen-fir forests) few roads exist and the primary mode of transport across the park is by boat. Lakeshore campsites were expected to be important sources of earthworm introduction. However a more accurate metric of the intensity of campsite use may provide more predictability for earthworm invasions. Additionally, in the beech-maple forests, increasing loamy soil texture in the E horizon (indicative of loamy soil conditions overall) was an indicator of the species assemblage and biomass of earthworms in a stand (Figure 6). Soil texture and moisture conditions have been shown to be important factors affecting earthworm distributions in other areas as well (Suárez et al. 2005, Shakir and Dindal 1997, Reich et al. unpublished data). Finally, earthworm biomass measures vary substantially among forest types (indicative of the dominant litter available) and will need to be considered in any landscape level analysis of earthworm invasion dynamics.

Earthworm species and biomass among different forest types

The same suite of earthworm species and assemblages were supported in the beech-maple and aspen-fir forests (Tables 1 and 2) as those seen in other forests across the Great Lakes region (Hale et al. 2005^a, Bohlen et al. 2004^a, Holdsworth et al. 2004, Scheu and McLean 1993, Morgenweck and Marshall 1982). However, in the most diverse earthworm assemblage, the total earthworm biomass supported by the beech-maple forest type (7.99 ADFg/m²) was double that of the aspen-fir forests (3.53 ADFg/m²) (Table 2). The same earthworm assemblage has been shown to support even higher total biomass (12 AFDg/m²) in sugar maple dominated forests of the Great Lakes region (Hale et al. 2005^a, Holdsworth et al. 2004). When a forest is strictly dominated by less palatable conifer species (e.g. spruce, hemlock, lodgepole pine) the earthworm assemblage may be limited to strictly epigeic species such as *Dendrobaena octaedra* and *Dendrodrilus rubidus* (Figure 6, Reich et al. 2005, Dymond et al. 1997, Terhivuo 1989).

Earthworm impacts on upper soil horizons

In both forest types, increasing earthworm species richness and total biomass were associated with declining O_{litter} and O_e horizon thickness and increasing A horizon thickness (Figure 3). The same pattern has also been seen in other habitats (Hale et al 2005^a, Shaw and Pawluk 1986, Nielsen and Hole 1964, Buntley and Papendick 1960). In sugar maple forests of Minnesota, the change in soil horizons begins when soil dwelling species arrive and reaches its maximum when all ecological groups of earthworms (epi-endogeic, endogeic and/or anecic) are present and the process of change in soil horizons continues as the earthworm population spreads through the forest (Hale et al. 2005^b, Hale 2004).

Earthworm total biomass of approximately 0.10 AFDg/m² may represent a threshold above which we begin to see proportional changes in forest soil characteristics as earthworm biomass increases (Figure 3). Total earthworm biomass this low is generally seen only when small epigeic species such as *Dendrobaena octaedra* and/or *Dendrodrilus rubidus* are the only species present (Table 2, Hale et al. 2005^b, Reich et al. 2005).

Earthworm impacts on forest plant communities

The understory plant compositional gradient across beech-maple stands is typical of a compositional gradient related to nitrogen availability and loamy soil conditions (Kotar et al. 2002, Host and Pregitzer 1992, Zak et al. 1986). Because we did not capture the natural variability of earthworm-free stands, we cannot determine if the increases in plant richness and shifts in composition associated with increasing earthworm biomass and species assemblage in beech-maple forests are a response by earthworms to the natural variability of the cover type (i.e., earthworms survive better in richer/wetter stands with loamy soils, so that is where we find them) or that they caused the increase in plant richness and cover. However, the latter seems unlikely given the number of plant species present in beech-maple stands with high earthworm biomass that have been shown to decline in response to earthworm invasions in sugar maple forests (Hale 2004).

Future research and monitoring priorities

One limitation to our efforts of assessing the impacts of exotic earthworm invasions across a range of forest cover types was our lack of information on earthworm-free stands. A comprehensive characterization of the natural variability of understory plant communities and forest floor and upper soil horizon characteristics in important forest types under earthworm-free conditions is needed. This would be particularly valuable for forest types that are commonly present across a wide range of soil conditions.

Forest stands on loamy soils with at least some hardwood component support larger and more diverse earthworm populations than forests stands on dry, sandy soils or those dominated by conifer species. The former are therefore more likely to experience greater impacts as a result of exotic earthworm invasion. Where resources for research and monitoring are limited, the richer hardwood forests should be the target of monitoring efforts.

Because different earthworm species and the amount of earthworm biomass strongly affects the impacts seen in many forests (Hale et al. 2005^b, Bohlen et al. 2004^a, Hale 2004, Gundale 2002, Migge et al. 1998), research and monitoring efforts related to exotic earthworms will be most effective if measures of species assemblage, diversity and relative biomass are used. Earthworm presence versus absence in a stand is clearly inadequate for predicting or monitoring exotic earthworm impacts. Because measurements of fresh and dry earthworm biomass can be significantly affected by the moisture conditions under which the earthworms are collected and their relative gut contents, ash-free dry mass is the best standardized measure of earthworm biomass. Ash-free dry biomass also allows for reliable comparison of biomass values among different locations and through time (Hale et al. 2004).

At this time, exotic earthworm populations have not detectably impacted the understory plant communities in either the beech-maple or aspen-fir forest stands included in this study. However, given that this is simply a snapshot in time of a limited number of forest stands, we still lack knowledge with respect to the impacts of the length of time since initial earthworm invasion. Therefore, we cannot currently say if there have been or will be any changes in plant community composition in response to earthworms. Substantial research has documented the potential of earthworms to change ecosystem level process and the structure and composition of plant and soil communities (Bohlen et al. 2004^b). The stands included in this study captured a wide range of earthworm species assemblages and total biomass and, with the addition of a set of

worm-free stands in each forest type, would make a good core set of stands for a continuing monitoring program in order to answer questions regarding the long-term dynamics and effects of earthworm invasion in these forests.

The greatest threat to areas minimally impacted by exotic earthworms is the establishment of new inoculation points in currently earthworm-free areas and the continued transport and establishment of additional species in areas where only a limited number of species are currently present (i.e., where *D. octaedra* is the only species present). Education, management, and land use efforts to protect earthworm-free areas and limit the continued establishment of additional species can be effective within the bounds of ecological reserves even if earthworms exist in close proximity to or in limited locations within the preserve. The natural spread of established earthworm populations is relatively slow, 5-10 m per year (Hale et al. 2005^a, Dymond et al. 1997, Marinissen and Van den Bosch 1992) which translates to ~200 years for 1 kilometer of earthworm spread. Therefore, continued assessment of exotic earthworm distributions within a reserve will be important for directing education, management, and policy decisions as they relate to exotic earthworm invasions.

These results, in addition to previous research on the impacts of exotic earthworm invasions in northern cold temperate forests (Hale et al. 2005^b, Bohlen et al. 2004^a, Hale 2004, McLean and Parkinson 1997) will help to direct future research and monitoring efforts and inform policy and land use decisions in the western Great Lakes region.

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Appendix 1. Beech-maple forest type: correlation coefficients (Pearson r) of common (present in ≥ 3 stands) understory plant species total cover with ordination axes and the number of stands in which each species was found (total $n = 20$).

Species	n	Axis 1	Axis 2	Axis 3
Herbaceous plants				
<i>Actaea pachypoda</i>	8	0.795	0.276	0.001
<i>Allium tricoccum</i>	7	0.421	0.106	0.21
<i>Aralia nudicaulis</i>	7	0.071	0.178	0.12
<i>Arisaema triphyllum</i>	5	0.155	0.011	0.02
<i>Athyrium filix-femina</i>	4	0.018	0.001	0.012
<i>Botrychium virginianum</i>	3	0.121	0.119	0.087
<i>Carex arctata</i>	9	0.005	0.007	0.346
<i>Carex communis</i>	3	0.17	0.014	0.017
<i>Carex deweyana</i>	6	0.601	0.108	0.053
<i>Carex intumescens</i>	6	0.033	0.007	0.012
<i>Carex leptoneura</i>	12	0.588	0.023	0.035
<i>Carex pedunculata</i>	6	0.192	0.092	0.025
<i>Carex</i> spp.	3	0.008	0.123	0.045
<i>Clintonia borealis</i>	9	0.122	0.166	0.103
<i>Coptis trifolia</i>	3	0.025	0.139	0.036
<i>Dentaria diphylla</i>	4	0.123	0.001	0.009
<i>Dicentra cucullaria</i>	3	0.335	0.027	0.028
<i>Dryopteris intermedia</i>	20	0.001	0.117	0.007
<i>Erythronium americanum</i>	15	0.016	0.002	0.275
<i>Galium triflorum</i>	4	0.128	0.008	0.124
<i>Gymnocarpium dryopteris</i>	4	0	0.022	0.047
<i>Hepatica americana</i>	3	0.172	0.23	0.046
<i>Lycopodium lucidulum</i>	17	0.128	0.124	0.012
<i>Lycopodium obscurum</i>	8	0.099	0.353	0.01
<i>Maianthemum canadense</i>	19	0.04	0.082	0.168
<i>Melica smithii</i>	3	0.232	0.206	0.163
<i>Milium effusum</i>	9	0.654	0.32	0.02
<i>Mitchella repens</i>	10	0.131	0.393	0.004
<i>Monotropa uniflora</i>	3	0.042	0.043	0.013
<i>Osmorhiza claytonii</i>	7	0.388	0.395	0.114
<i>Oxalis acetosella</i>	6	0.066	0.001	0.003
<i>Polygonatum pubescens</i>	13	0.482	0.352	0.088

Appendix 1. continued	n	Axis 1	Axis 2	Axis 3
<i>Smilacina racemosa</i>	10	0.29	0.114	0.001
<i>Streptopus roseus</i>	19	0.501	0.317	0.054
<i>Trientalis borealis</i>	12	0.189	0.089	0.029
<i>Trillium cernuum</i>	5	0.347	0.017	0.057
<i>Viola canadensis</i>	10	0.548	0.386	0.026
<i>Viola pubescens</i>	5	0.302	0.042	0.043
<i>Viola sororia</i>	6	0.09	0	0.168
Tree Seedlings				
<i>Abies balsamea</i>	12	0.024	0.152	0.045
<i>Acer pensylvanicum</i>	10	0.524	0.076	0.102
<i>Acer rubrum</i>	11	0.331	0.278	0.084
<i>Acer saccharum</i>	20	0.199	0.341	0.229
<i>Amelanchier</i> spp.	10	0.002	0.323	0.004
<i>Betula alleghaniensis</i>	3	0.104	0.047	0.014
<i>Corylus cornuta</i>	4	0.002	0.001	0.071
<i>Fagus grandifolia</i>	20	0.01	0.161	0.011
<i>Lonicera canadensis</i>	14	0.014	0.041	0.019
<i>Pinus strobus</i>	3	0.01	0.175	0.009
<i>Prunus serotina</i>	6	0	0	0.067
<i>Prunus virginiana</i>	3	0.166	0.225	0.204
<i>Sambucus racemosa</i>	8	0.381	0.324	0
<i>Sorbus decora</i>	10	0.035	0.145	0.257
<i>Taxus canadensis</i>	6	0.075	0.064	0.188
<i>Tsuga canadensis</i>	3	0.047	0.079	0.024
Small Saplings and Shrubs				
<i>Abies balsamea</i>	17	0.004	0.007	0.418
<i>Acer pensylvanicum</i>	7	0.362	0.078	0.246
<i>Acer pensylvanicum</i>	7	0.362	0.078	0.246
<i>Acer rubrum</i>	3	0.006	0.004	0.022
<i>Acer saccharum</i>	18	0.009	0.105	0.066
<i>Amelanchier</i> spp.	3	0.04	0.153	0
<i>Betula alleghaniensis</i>	3	0.102	0.008	0.006
<i>Fagus grandifolia</i>	20	0.085	0.407	0.448
<i>Lonicera canadensis</i>	5	0.132	0.04	0.181
<i>Ostrya virginiana</i>	3	0.207	0.045	0.144
<i>Sambucus racemosa</i>	4	0.436	0.057	0.158

Appendix 1. continued	n	Axis 1	Axis 2	Axis 3
Large Saplings and Shrubs				
<i>Abies balsamea</i>	11	0.045	0.23	0.153
<i>Acer pensylvanicum</i>	4	0.188	0.204	0.232
<i>Acer rubrum</i>	6	0.227	0.186	0.065
<i>Acer saccharum</i>	20	0.216	0.038	0.012
<i>Betula alleghaniensis</i>	4	0.006	0.226	0.053
<i>Fagus grandifolia</i>	18	0.311	0.44	0.045

Appendix 2. Beech-maple forest type: correlation coefficients (Pearson r) of environmental variables and canopy tree species basal area with ordination axes.

Environmental Variable	Axis 1	Axis 2	Axis 3
Earthworms			
Total earthworm biomass	0.558	0.021	0.021
<i>Aporrectodea</i> species biomass	0.599	0.2	0.001
<i>D. octaedra</i> biomass	0.311	0.003	0.001
<i>L. rubellus</i> biomass	0.202	0.047	0.05
<i>L.</i> juvenile biomass	0.566	0.11	0.015
<i>L. terrestris</i> biomass	0.324	0.052	0.077
earthworm richness	0.567	0.088	0
Plant measures			
Total basal area	0.108	0.009	0.099
Tree species basal area			
<i>Abies balsamea</i>	0	0.041	0.019
<i>Acer pensylvanicum</i>	0.026	0.157	0.08
<i>Acer rubrum</i>	0.083	0.42	0.042
<i>Acer saccharum</i>		0.274	0.231
<i>Betula alleghaniensis</i>	0.061	0.274	0.06
<i>Betula papyrifera</i>	0.004	0.054	0.042
<i>Fagus grandifolia</i>	0.039	0.006	0.001
<i>Ostrya virginiana</i>	0.021	0.2	0.033
<i>Picea glauca</i>	0.033	0.074	0.006
<i>Pinus resinosa</i>	0.003	0.302	0
<i>Pinus strobus</i>	0.128	0.009	0.105
<i>Populus grandidentata</i>	0.024	0.138	0.061
<i>Populus tremuloides</i>	0.07	0.029	0.207
<i>Prunus serotina</i>	0.012	0	0.16
<i>Prunus virginiana</i>	0.021	0.2	0.033
<i>Thuja occidentalis</i>	0.002	0.033	0.065
<i>Tsuga canadensis</i>	0.239	0.005	0.131
Species Richness			
All vegetation	0.322	0.003	0.097
Herbaceous plants	0.609	0.043	0.001
Tree Seedlings	0.005	0.111	0.275
Small Saplings and Shrubs	0.076	0.007	0.219
Large Saplings and Shrubs	0.064	0.307	0.153

Appendix 2. continued	Axis 1	Axis 2	Axis 3
Shannon (H') Species Diversity			
All vegetation	0.137	0.021	0.305
Herbaceous plants	0.268	0.015	0.009
Tree Seedlings	0.219	0.226	0.358
Small Saplings and Shrubs	0.073	0.011	0.241
Large Saplings and Shrubs	0.028	0.403	0.202
Total Percent Cover			
All vegetation	0.062	0.109	0.328
Herbaceous plants	0.16	0.406	0.017
Tree Seedlings	0.004	0.01	0.278
Small Saplings and Shrubs	0.088	0.219	0.555
Large Saplings and Shrubs	0.257	0.558	0.131
Soil Horizons			
O _{litter} horizon thickness (cm)	0.03	0.048	0.068
O _e horizon thickness (cm)	0.376	0.078	0.002
O _a horizon thickness (cm)	0.035	0.016	0.033
Total O horizon thickness (cm)	0.181	0.005	0.039
A horizon thickness (cm)	0.45	0.05	0
A horizon texture class	0.189	0.019	0.04
E horizon texture class	0.279	0.17	0
Distance to Human Development (m)			
Distance to nearest trail	0.095	0.04	0
Distance to nearest road	0.012	0.016	0.022
Distance to nearest campsite	0	0.27	0.01
Distance to next vegetation type	0.058	0.011	0.088
Distance to nearest stream	0.063	0.071	0.204
Distance to 2nd nearest stream	0.033	0.065	0.248
Distance to 3rd nearest stream	0.032	0.096	0.262
Distance to nearest lake	0.009	0.013	0.279
Distance to 2nd nearest lake	0.079	0	0.316
Distance to 3rd nearest lake	0.103	0.006	0.259

Appendix 3. Aspen-fir forest type: correlation coefficients (Pearson r) of common (present in ≥ 3 stands) understory plant species total cover with ordination axes and the number of stands each species was found (total $n = 20$).

Species	n	Axis 1	Axis 2	Axis 3
Herbaceous plants				
<i>Actaea rubra</i>	6	0.057	0.244	0.043
<i>Anemone quinquefolia</i>	6	0.001	0.086	0.276
<i>Apocynum androsaemifolium</i>	3	0	0.009	0
<i>Aralia nudicaulis</i>	20	0	0.147	0.049
<i>Asarum canadense</i>	3	0.214	0.012	0.126
<i>Aster ciliolatus</i>	5	0.073	0.35	0.017
<i>Aster macrophyllus</i>	20	0.054	0.011	0.093
<i>Athyrium filix-femina</i>	12	0.236	0	0.526
<i>Brachyelytrum erectum</i>	8	0.039	0.079	0.101
<i>Carex arctata</i>	14	0.126	0.125	0.036
<i>Carex brunnescens</i>	11	0.21	0.02	0.172
<i>Carex deweyana</i>	7	0.01	0.506	0.035
<i>Carex gracillima</i>	5	0.155	0.03	0.055
<i>Carex intumescens</i>	8	0.028	0.094	0.072
<i>Carex peckii</i>	3	0.183	0.057	0.009
<i>Carex pedunculata</i>	10	0.257	0.167	0.035
<i>Carex pensylvanica</i>	3	0	0	0.09
<i>Carex</i> spp.	13	0.002	0.331	0.032
<i>Circaea alpina</i>	6	0.14	0.517	0.001
<i>Clintonia borealis</i>	20	0.035	0	0.001
<i>Coptis trifolia</i>	8	0.024	0.001	0.247
<i>Cornus canadensis</i>	20	0.18	0.264	0.043
<i>Dryopteris carthusiana</i>	14	0.105	0	0
<i>Equisetum arvense</i>	3	0.09	0.154	0.02
<i>Equisetum pratense</i>	3	0.001	0.001	0.007
<i>Equisetum sylvaticum</i>	12	0.205	0.064	0.001
<i>Fragaria virginiana</i>	15	0.42	0	0.035
<i>Galium triflorum</i>	16	0.404	0.046	0.307
<i>Gymnocarpium dryopteris</i>	11	0.009	0.117	0.248
<i>Lactuca canadensis</i>	3	0.148	0.007	0.075
<i>Lathyrus ochroleucus</i>	16	0.229	0.292	0
<i>Linnaea borealis</i>	14	0.098	0.019	0.001

Appendix 3. continued	n	Axis 1	Axis 2	Axis 3
<i>Luzula multiflora</i>	3	0.157	0.07	0
<i>Lycopodium annotinum</i>	7	0.13	0.318	0
<i>Lycopodium clavatum</i>	17	0.533	0.141	0.06
<i>Lycopodium complanatum</i>	3	0.37	0	0.129
<i>Lycopodium lucidulum</i>	9	0.005	0.043	0.003
<i>Lycopodium obscurum</i>	18	0.1	0.292	0.032
<i>Lycopodium tristachyum</i>	3	0.074	0.003	0.068
<i>Lycopus uniflorus</i>	7	0.02	0.272	0
<i>Maianthemum canadense</i>	20	0.023	0.371	0.002
<i>Melampyrum lineare</i>	8	0.304	0.333	0.186
<i>Mitella nuda</i>	8	0.251	0.212	0.032
<i>Oryzopsis asperifolia</i>	20	0.01	0.191	0.044
<i>Osmunda claytoniana</i>	3	0.077	0.169	0.26
<i>Petasites frigidus</i> var. <i>palmatus</i>	9	0.167	0.028	0.006
<i>Poa interior</i>	4	0.03	0.245	0.104
<i>Poa palustris</i>	6	0.001	0.16	0.101
<i>Polygonum cilinode</i>	7	0.077	0.169	0.007
<i>Polypodium virginianum</i>	4	0.017	0.039	0.063
<i>Pteridium aquilinum</i>	17	0.011	0.13	0.051
<i>Pyrola chlorantha</i>	7	0.191	0.001	0.651
<i>Schizachne purpurascens</i>	14	0.006	0.015	0.026
<i>Solidago canadensis</i>	4	0.09	0.271	0.062
<i>Streptopus roseus</i>	20	0.031	0.263	0.028
<i>Trientalis borealis</i>	20	0.126	0.022	0
<i>Trillium cernuum</i>	3	0.149	0.003	0.1
<i>Viola canadensis</i>	11	0.081	0.322	0.07
<i>Viola renifolia</i>	5	0.015	0.115	0.059
<i>Viola sororia</i>	9	0.004	0.13	0.001
Tree Seedlings				
<i>Abies balsamea</i>	20	0.019	0.004	0.02
<i>Acer rubrum</i>	20	0.003	0.059	0.049
<i>Acer spicatum</i>	17	0.119	0.603	0.207
<i>Alnus incana</i> ssp. <i>rugosa</i>	5	0.082	0.37	0.048
<i>Amelanchier</i> spp.	19	0.056	0.022	0.13
<i>Betula papyrifera</i>	15	0.006	0.078	0.133
<i>Cornus alternifolia</i>	7	0.126	0.063	0.023

Appendix 3. continued	n	Axis 1	Axis 2	Axis 3
<i>Cornus rugosa</i>	4	0.17	0.019	0.082
<i>Cornus stolonifera</i>	3	0.064	0.173	0.006
<i>Corylus cornuta</i>	19	0.009	0.01	0.206
<i>Diervilla lonicera</i>	19	0.013	0.002	0.022
<i>Fraxinus nigra</i>	11	0.211	0.097	0.211
<i>Fraxinus pennsylvanica</i>	5	0.111	0.023	0.021
<i>Lonicera canadensis</i>	19	0.05	0.205	0.015
<i>Lonicera hirsuta</i>	15	0.196	0.037	0.082
<i>Picea glauca</i>	14	0.108	0.304	0.014
<i>Picea mariana</i>	10	0.25	0.239	0.091
<i>Pinus strobus</i>	18	0.094	0.076	0.202
<i>Populus grandidentata</i>	6	0.233	0	0.258
<i>Populus tremuloides</i>	20	0.046	0.01	0.061
<i>Prunus virginiana</i>	6	0.294	0.029	0.013
<i>Quercus macrocarpa</i>	7	0.149	0.157	0.002
<i>Quercus rubra</i>	17	0.13	0.001	0.254
<i>Ribes hirtellum</i>	5	0.089	0.081	0.136
<i>Ribes triste</i>	10	0.215	0.122	0.056
<i>Rosa acicularis</i>	14	0.058	0.038	0.037
<i>Rubus idaeus</i> ssp. <i>strigosus</i>	15	0.102	0.313	0.004
<i>Rubus pubescens</i>	18	0.405	0.006	0.083
<i>Sorbus decora</i>	8	0.432	0.013	0.185
<i>Tilia americana</i>	3	0.007	0.262	0.091
<i>Vaccinium angustifolium</i>	18	0.409	0.005	0.009
<i>Vaccinium myrtilloides</i>	16	0.03	0.007	0.41
<i>Viburnum rafinesquianum</i>	11	0.166	0.05	0.025
Small Saplings and Shrubs				
<i>Abies balsamea</i>	18	0.05	0.072	0.057
<i>Acer rubrum</i>	19	0.067	0.013	0.045
<i>Alnus incana</i> ssp. <i>rugosa</i>	5	0.007	0.313	0
<i>Amelanchier</i> spp.	18	0.062	0.006	0.218
<i>Betula papyrifera</i>	15	0.027	0.091	0.049
<i>Cornus alternifolia</i>	3	0.013	0.114	0.066
<i>Cornus rugosa</i>	6	0.136	0.047	0
<i>Corylus cornuta</i>	20	0.018	0.119	0.002
<i>Fraxinus nigra</i>	8	0.146	0.003	0.168

Appendix 3. continued	n	Axis 1	Axis 2	Axis 3
<i>Fraxinus pennsylvanica</i>	4	0.153	0.057	0
<i>Lonicera canadensis</i>	20	0.107	0.539	0.099
<i>Picea glauca</i>	15	0.055	0.114	0.248
<i>Picea mariana</i>	8	0.432	0.127	0.038
<i>Pinus strobus</i>	12	0.125	0.049	0.608
<i>Populus grandidentata</i>	8	0.086	0.005	0.142
<i>Populus tremuloides</i>	20	0.083	0.001	0.227
<i>Quercus macrocarpa</i>	5	0.146	0.05	0.077
<i>Quercus rubra</i>	9	0.011	0.07	0.392
<i>Ribes triste</i>	3	0.068	0.012	0.042
<i>Rubus idaeus</i> ssp. <i>strigosus</i>	7	0.139	0.314	0.048
<i>Vaccinium myrtilloides</i>	3	0.063	0.082	0.067
<i>Viburnum rafinesquianum</i>	9	0.245	0.02	0.112
Large Saplings and Shrubs				
<i>Abies balsamea</i>	20	0.002	0.014	0.056
<i>Acer rubrum</i>	15	0.037	0.011	0.018
<i>Acer spicatum</i>	10	0.212	0.415	0.009
<i>Alnus incana</i> ssp. <i>rugosa</i>	5	0.003	0.354	0.004
<i>Amelanchier</i> spp.	8	0.322	0.001	0.108
<i>Betula papyrifera</i>	19	0.06	0.026	0.01
<i>Corylus cornuta</i>	12	0.368	0.045	0.145
<i>Fraxinus nigra</i>	3	0.149	0.021	0.001
<i>Fraxinus pennsylvanica</i>	3	0.083	0.221	0.001
<i>Picea glauca</i>	15	0.019	0.009	0.25
<i>Picea mariana</i>	8	0.274	0.181	0.023
<i>Pinus strobus</i>	5	0.001	0.042	0.414
<i>Populus grandidentata</i>	5	0.025	0.093	0.025
<i>Populus tremuloides</i>	20	0.133	0.001	0.153
<i>Quercus macrocarpa</i>	3	0.038	0.318	0
<i>Quercus rubra</i>	3	0.066	0.021	0.329

Appendix 4. Aspen-fir Forest type: correlation coefficients (Pearson r) of environmental variables with ordination axis.

Environmental Variable	Axis 1	Axis 2	Axis 3
Earthworms			
Total earthworm biomass	0.021	0.001	0.001
<i>Aporrectodea</i> species biomass	0.007	0.043	0.016
<i>D. octaedra</i> biomass	0.008	0.088	0.04
<i>L. rubellus</i> biomass	0.163	0.067	0.01
<i>L.</i> juvenile biomass	0.069	0.05	0
<i>L. terrestris</i> biomass	0.006	0.009	0.007
earthworm richness	0.067	0.005	0.004
Plant measures			
Total basal area	0.056	0.053	0
Tree species basal area			
<i>Abies balsamea</i>	0.289	0.102	0.085
<i>Acer rubrum</i>	0.045	0.023	0.129
<i>Betula papyrifera</i>	0.041	0.211	0.031
<i>Fraxinus nigra</i>	0.046	0.01	0.061
<i>Picea glauca</i>	0.256	0.053	0.1
<i>Picea mariana</i>	0.148	0.001	0.019
<i>Pinus banksiana</i>	0.264	0.001	0
<i>Pinus resinosa</i>	0.048	0.043	0.012
<i>Pinus strobus</i>	0.008	0.003	0.246
<i>Populus grandidentata</i>	0.342	0.002	0.149
<i>Populus tremuloides</i>	0.207	0.006	0.029
<i>Quercus macrocarpa</i>	0.004	0.053	0.185
<i>Quercus rubra</i>	0.003	0.057	0.276
<i>Thuja occidentalis</i>	0	0.084	0.003
Species Richness			
All vegetation	0.652	0.012	0.022
Herbaceous plants	0.529	0.003	0.105
Tree Seedlings	0.316	0.001	0.057
Small Saplings and Shrubs	0.103	0.017	0.148
Large Saplings and Shrubs	0.292	0.039	0.045
Shannon (H') Species Diversity			
All vegetation	0.059	0.046	0.001
Herbaceous plants	0.032	0.017	0.135

Appendix 4. continued	Axis 1	Axis 2	Axis 3
Tree Seedlings	0.078	0.007	0.01
Small Saplings and Shrubs	0.166	0.023	0.007
Large Saplings and Shrubs	0.19	0.005	0.085
Total Percent Cover			
All vegetation	---	---	---
Herbaceous plants	0.014	0.233	0.001
Tree Seedlings	0	0.016	0
Small Saplings and Shrubs	0.082	0.17	0.137
Large Saplings and Shrubs	0.142	0.201	0.005
Soil Horizons			
O _{litter} horizon thickness (cm)	0.038	0.019	0.012
O _e horizon thickness (cm)	0.004	0.002	0.113
O _a horizon thickness (cm)	0.009	0.161	0.193
Total O horizon thickness (cm)	0	0.125	0.017
A horizon thickness (cm)	0.026	0.141	0.029
A horizon texture class	0.039	0.151	0.006
E horizon texture class	0.002	0.011	0
Distance to Human Development (m)			
Distance to nearest trail	0.164	0.03	0.006
Distance to nearest road	0.32	0	0.135
Distance to nearest campsite	0.03	0.044	0.057
Distance to next vegetation type	0.069	0.041	0.01
Distance to nearest lake	0.028	0.001	0.29
Distance to nearest stream	0.004	0.021	0.007
Distance to 2nd nearest stream	0	0.062	0.006
Distance to 3rd nearest stream	0.004	0.069	0.01